

## Symposium 18: The Alternating Access Mechanism in the Era of Transporter Structures

### 2786-Symp

#### Alternatives in Alternating Access

Christopher Miller.

Brandeis University, Waltham, MA, USA.

### 2787-Symp

#### The old Man and the Membrane

H. Ronald Kaback.

Univ of CA, Los Angeles, Los Angeles, CA, USA.

Lactose permease of *Escherichia coli* (LacY) is highly dynamic, and sugar binding causes closing of a large inward-facing cavity with opening of a wide outward-facing hydrophilic cavity. Therefore, lactose/H<sup>+</sup> symport via LacY very likely involves a global conformational change that allows alternating access of single sugar- and H<sup>+</sup>-binding sites to either side of the membrane. This presentation will review in camera the various biochemical/biophysical approaches that provide experimental evidence for the alternating access mechanism.

### 2788-Symp

#### Structure of Multidrug Resistance Transporters

Geoffrey Chang, Andrew Ward, Rupali Aggarwal, Alexandra Caya.

The Scripps Research Institute, La Jolla, CA, USA.

P-glycoprotein (Pgp) detoxifies cells by exporting hundreds of chemically unrelated toxins but has been implicated in multidrug resistance in the treatment of cancers. Substrate promiscuity is a hallmark of Pgp activity, thus a structural description of polyspecific drug-binding is important for the rational design of anticancer drugs and MDR inhibitors. The x-ray structure of apo-Pgp at 3.8 Å reveals an internal cavity of ~6,000 Å<sup>3</sup> with a 30 Å separation of the two nucleotide binding domains (NBD). Two additional Pgp structures with cyclic peptide inhibitors demonstrate distinct drug binding sites in the internal cavity capable of stereo-selectivity that is based on hydrophobic and aromatic interactions. Apo- and drug-bound Pgp structures have portals open to the cytoplasm and the inner leaflet of the lipid bilayer for drug entry. The inward-facing conformation represents an initial stage of the transport cycle that is competent for drug binding. We will present our latest findings on P-glycoprotein and present strategy on obtaining other conformations, extending the diffraction resolution, and new co-crystal structures with inhibitors/drugs.

## Symposium 19: Molecular Motors and the Cytoskeleton: Moving to the Boundaries

### 2790-Symp

#### To Cut or not to Cut?: Physically Regulating Microtubule Severing Enzymes

Jennifer Ross.

University of Massachusetts, Amherst, MA, USA.

Microtubule-severing enzymes are AAA (ATPases associated with cellular activities) proteins that remove tubulin dimers from the microtubule lattice. Severing proteins are known to remodel the cytoskeleton during interphase and mitosis, and are required in proper axon morphology and mammalian bone and cartilage development. We have performed the first single molecule imaging to determine where and how severing enzymes act to cut microtubules. We have focused on the original member of the group, katanin, and the newest member, fidgetin to compare their biophysical activities *in vitro*. We find that, at lower concentrations, both katanin and fidgetin can depolymerize microtubules by removing terminal dimers. Katanin preferentially removes dimers from the plus-end, while fidgetin removes from the minus-end. This activity reflects their cellular localization and activity, where katanin is localized to the cell cortex and fidgetin at the microtubule-organizing center. At higher concentrations, both katanin and fidgetin can sever microtubules, but katanin cuts cleanly through, while fidgetin appear to remove long strips of protofilaments, without a clean break. Further, we find that katanin and fidgetin prefer different types of tubulin to bind and sever. These studies reveal the physical regulation schemes to control severing activity in cells, and ultimately regulate cytoskeletal architecture.

### 2791-Symp

#### Invited Speaker

Michael Welte.

Brandeis University, Waltham, MA, USA.

### 2792-Symp

#### Active Patterning and Contractile Dynamics in Actin Networks Driven by Myosin Motors

Gijsje H. Koenderink<sup>1</sup>, Marina Soares Silva<sup>1</sup>, Martin Depken<sup>2</sup>,

Bjorn Stuhmann<sup>1</sup>, Fred C. MacKintosh<sup>2</sup>.

<sup>1</sup>FOM Institute AMOLF, Amsterdam, Netherlands, <sup>2</sup>Free University

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Self-organized contractile arrays of actin filaments and myosin motors drive cell division, migration, and tissue morphogenesis. Biophysical studies have provided many insights into the mechanisms of force production by individual motor molecule. However, a mechanistic explanation of collective self-assembly into force-generating arrays is still lacking. We studied how the collective activity of myosin motors organizes actin filaments into contractile structures in a simplified model system devoid of biochemical regulation. We showed that myosin organizes actin into contractile arrays by a 3-stage process. First, the actin filaments mediate the formation of dense foci by active motor transport and motor coalescence. The myosin foci then accumulate actin filaments in a disordered cloud around them, and these actomyosin condensates finally merge into superaggregates by contractile coalescence. We propose that the origin of this multistage aggregation is the highly nonlinear load response of actin filaments, which can support large tensions but buckle already under piconewton-compressive loads. Since the motor generated forces well exceed this buckling threshold, buckling is induced by the elastic resistance of connected actin networks to filament sliding. We furthermore mapped the spatiotemporal characteristics of the motor-induced contractility by tracking embedded particles, and found that myosin induces long-range, but localized, contractile fluctuations. The contractile dynamics and actomyosin superaggregates closely mimic observations *in vivo*. However, the localization and turnover of actomyosin arrays and directed cortical flows likely require an interplay of collective self-organization and biochemical regulation.

### 2793-Symp

#### Myosin Molecular Motors: Transporting Cargo in All Directions

David Warshaw.

University of Vermont College of Medicine, Burlington, VT, USA.

## Platform AW: Cell and Bacterial Mechanics & Motility II

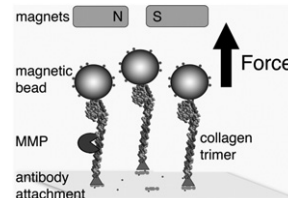
### 2794-Plat

#### Mechanical Load Induces a 100-Fold Increase in the Rate of Collagen Proteolysis by MMP-1

Arjun S. Adhikari, Jack Chai, Alexander R. Dunn.

Stanford University, Stanford, CA, USA.

Both mechanotransduction and extracellular matrix (ECM) proteolytic remodeling are critical during embryonic development and in disease progression. Although mechanical stress is known to profoundly influence ECM remodeling, its effect on ECM degradation by matrix metalloproteinases (MMPs) is largely unexplored. We used a single-molecule magnetic tweezers assay to study the effect of force on collagen proteolysis by MMP-1. We show that the application of ~10 pN in extensional force (far less than cell traction forces) causes a ~100-fold increase in proteolysis rates. Our results support a mechanistic model in which the collagen triple helix unwinds prior to proteolysis. The data and resulting model predict that cell-generated traction forces may dramatically increase localized collagen proteolysis, suggesting that cells may use mechanical force to regulate proteolytic ECM remodeling.



### 2795-Plat

#### Cell Shape Dynamics: from Waves to Motion

Wolfgang Losert<sup>1</sup>, Meghan Driscoll<sup>1</sup>, Colin McCann<sup>1</sup>, John Fourkas<sup>1</sup>,

Carole Parent<sup>2</sup>.

<sup>1</sup>University of Maryland, College Park, MD, USA, <sup>2</sup>National Cancer

Institute, Bethesda, MD, USA.

We investigate the dynamic changes in cell shapes during cell motility. Using active contour algorithms, we demonstrate the existence of wave-like dynamic shape changes during the migration of *Dictyostelium discoideum*, a model system for the study of chemotaxis. Cell shapes have regions of high boundary curvature that propagate from the leading edge toward the back along usually alternating sides of the cell. To study the relationship between these curvature